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Role of Protein Tyrosine Phosphatases in Regulating the Immune System: Implications for Chronic Intestinal Inflammation

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Abstract: Current hypothesis suggests that genetic, immunological, and bacterial factors contribute essentially to the pathogenesis of inflammatory bowel disease. Variations within the gene loci encoding protein tyrosine phosphatases (PTPs) have been associated with the onset of inflammatory bowel disease. PTPs modulate the activity of their substrates by dephosphorylation of tyrosine residues and are critical for the regulation of fundamental cellular signaling processes. Evidence emerges that expression levels of PTPN2, PTPN11, and PTPN22 are altered in actively inflamed intestinal tissue. PTPN2 seems to be critical for protecting intestinal epithelial barrier function, regulating innate and adaptive immune responses and finally for maintaining intestinal homeostasis. These observations have been confirmed in PTPN2 knockout mice in vivo. Those animals are clearly more susceptible to intestinal and systemic inflammation and feature alterations in innate and adaptive immune responses. PTPN22 controls inflammatory signaling in lymphocytes and mononuclear cells resulting in aberrant cytokine secretion pattern and autophagosome formation. PTPN22 deficiency in vivo results in more severe colitis demonstrating the relevance of PTPN22 for intestinal homeostasis in vivo. Of note, loss of PTPN22 promotes mitogen-activated protein kinase-induced cytokine secretion but limits secretion of nuclear factor κ B-associated cytokines and autophagy in mononuclear cells. Loss of PTPN11 is also associated with increased colitis severity in vivo. In summary, dysfunction of those PTPs results in aberrant and uncontrolled immune responses that result in chronic inflammatory conditions. This way, it becomes more and more evident that dysfunction of PTPs displays an important factor in the pathogenesis of chronic intestinal inflammation, in particular inflammatory bowel disease.

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Key Words: PTPN2 and IBD, PTPN22 and IBD, PTPN11 and IBD

Current hypothesis suggests that genetic, immunological, and bacterial factors contribute essentially to the pathogenesis of inflammatory bowel disease (IBD). An epithelial barrier defect, coupled with a dysfunctional immune response of the innate and the acquired immune system to commensal flora results in either excessive upregulation or impaired downregulation of inflammatory events, what finally drives the development of chronic intestinal inflammation.¹ The predispositions are genetically determined, and genome-wide association studies (GWAS) identified variations in

about 160 gene loci being associated with IBD.² Many of the identified risk genes are critically involved in bacterial recognition, induction of antimicrobial factors, activation and modulation of innate and adaptive immune responses, and the maintenance of intestinal epithelial barrier function.

Phosphorylation and dephosphorylation of specific amino acid residues, in particular tyrosine residues, represent a fundamental mechanism for the activation and inactivation of intracellular signaling molecules. Dephosphorylation is performed by a large number of different protein phosphatases. One important family of such proteins is protein tyrosine phosphatases (PTPs). Members of this family play an essential role in the regulation of critical cell signaling events, i.e., proliferation, differentiation, and cell survival. Recent studies demonstrated a pivotal role for PTPs in the regulation of inflammatory and antibacterial responses.³

In this review, we will summarize the most recent knowledge about the role of PTPNs in the pathogenesis of chronic intestinal inflammation, in particular IBD.

PTP NON-RECEPTOR TYPE 22

In the past decade, GWAS revealed a strong association between 2 different single nucleotide polymorphisms (SNPs) in the gene locus encoding protein tyrosine phosphatase non-receptor type 22 (PTPN22) and several inflammatory disorders, including the IBD subforms, Crohn's disease (CD)^{2,4} and ulcerative colitis (UC),^{2,4} systemic lupus erythematosus,⁵ rheumatoid

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arthritis,^{6,7} type 1 diabetes (T1D),⁸ and others.⁹ One disease-associated SNP (C1858>T; SNP ID: rs2476601) results in the substitution of Arginine 620 to a tryptophan residue in the protein product (referred to as 620W variant), whereas in the other SNP (G788>C; SNP ID: rs33996649), arginine 263 is substituted with a glutamine residue (referred to as 263Q variant).

Arginine 263 lies within the catalytic domain of the protein, and substitution with a glutamine residue results in decreased phosphatase activity.¹⁰ Of note, presence of the 263Q variant reduces the risk to develop systemic lupus erythematoses and rheumatoid arthritis^{10,11} and protects from UC onset.⁴ PTPN22 620W in contrast is associated with increased risk for developing rheumatoid arthritis,^{6,7} systemic lupus erythematoses,⁵ and T1D⁸ but reduces the risk to develop CD.⁴ Initial studies by Vang et al¹² demonstrated that presence of this genetic variation results in a gain-of-function PTPN22 variant causing inhibition of the T-cell receptor signaling cascade. Furthermore, this variant features increased *in vitro* dephosphorylation capacity,¹² possibly resulting from reduced PTPN22 phosphorylation at an inhibitory residue.^{13,14} In contrast, one study using mice that express the disease-associated PTPN22 variant, suggested that the PTPN22 619W variant (the murine ortholog of human PTPN22 620W) results in reduced protein stability and therefore in a loss-of-function effect of this variant.¹⁵ However, this finding could not be confirmed in other studies using an independently generated PTPN22 619W knockin strain.¹⁶

In vivo silencing of PTPN22 by siRNA interference caused phenotypically opposite effects on B-cell differentiation when compared with the effects of the presence of the PTPN22 620W allele in humans.¹⁷ This further supports the gain-of-function hypothesis. Wang et al¹⁸ recently proposed that the 620W variant might be an altered function, rather than a loss- or gain-of-function variant, proposing altered substrate specificity of the variant as possible mechanism for the observed effects.¹⁸ Nevertheless, in general, the PTPN22 620W variant is nowadays regarded as a gain-of-function variant.

PTPN22 in Lymphocytes

PTPN22 is expressed in both, myeloid and lymphoid immune cells, but barely present in nonhematopoietic cell types.¹⁹ Given the strong association with inflammatory diseases, several studies addressed the role of PTPN22 in immune cell signaling. In T cells, PTPN22 activity attenuates T-cell antigen receptor (TCR) signaling by interacting with and dephosphorylating the TCR-associated kinases lymphocyte-specific protein tyrosine kinase (Lck) and zeta-chain associated protein (ZAP)-70.^{20,21} Presence of the gain-of-function variant results in reduced T-cell receptor signaling in T1D patients and healthy individuals,¹⁴ whereas PTPN22-deficient T cells are hyperresponsive to TCR ligation.²² PTPN22-deficient mice, in contrast, display increased levels of effector and memory T cells but interestingly do not show signs of autoimmunity.²² This observation was explained in additional studies, which revealed that PTPN22-deficient regulatory T cells show enhanced suppressor functions and thereby are able to

counteract the increased T cell activation found in PTPN22-deficient effector T cells.²³

Presence of the PTPN22 620W variant leads to an increased frequency of autoreactive B-cell clones in healthy individuals^{24,25} and T1D patients.²⁵ Several studies argue that this is the result of B-cell intrinsic defects,¹⁶ such as enhanced survival of autoreactive B cells at the transition from immature to naive B cells^{24,25} due to changed breakpoint cluster region signaling.^{24,25} A more recent study however claims that this effect might result from changes in follicular Th-cell activation, which drives altered activation of B cells in germinal centers, rather than from a B-cell intrinsic effect.²⁶

PTPN22 in Myeloid Immune Cells

Most studies addressing PTPN22 function have been performed in lymphocytes, but PTPN22 expression is very high in other immune cell types as well, including neutrophils, macrophages, and dendritic cells.^{15,19} We showed that loss of PTPN22 in monocytes results in profound changes in interferon gamma (IFN- γ)-induced signaling with reduced signal transducer and activator of transcription (STAT) 1, but strongly enhanced p38 mitogen-activated protein kinase (MAPK) activation, ultimately resulting in drastically enhanced interleukin (IL)-6 secretion together with reduced IL-12 levels.²⁷ Furthermore, loss of PTPN22 also resulted in an altered response to the bacterial wall product, muramyl dipeptide (MDP), with enhanced autophagy induction but also increased secretion of proinflammatory IL-6 and IL-8²⁷; hence, loss of PTPN22 in myeloid immune cells results in an increase in the secretion of proinflammatory cytokines (Fig. 1).

Wang et al¹⁸ demonstrated that presence of the PTPN22 620W variant results in impaired type-1 IFN production on TLR4 ligation, and loss of PTPN22, or the presence of the 620W variant, promotes differentiation of monocytes into proinflammatory M1

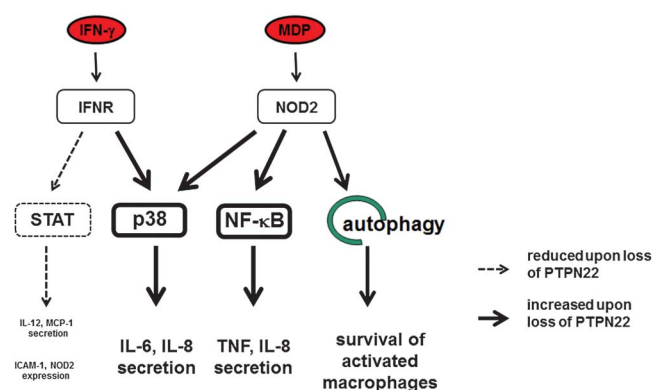


FIGURE 1. PTPN22 controls IFN- γ - and MDP-induced signaling cascades. Loss of PTPN22 promotes p38 MAPK and canonical NF κ B activation and increases MDP-induced autophagy. However, loss of PTPN22 results in decreased STAT activity. Taken together, this results in a highly imbalanced cytokine secretion pattern. ICAM-1, intercellular adhesion molecule 1; IFN, interferon; IFNR, interferon receptor; MCP-1, monocyte chemoattractant protein 1; NF κ B, nuclear factor κ B; NOD2, nucleotide oligomerization containing protein 2.

macrophages.²⁸ Dendritic cells from PTPN22-deficient mice expressed higher levels of the costimulatory molecule CD40 and induced higher proliferation of OT-II T cells in *in vitro* coculture experiments.¹⁵

PTPN22 in Patients with IBD

The PTPN22 gain-of-function variant is regarded as a general autoimmune risk allele, as it is associated with increased risk to develop several autoimmune disorders.⁹ Interestingly, the gain-of-function variant at the same time results in a reduced risk to develop CD.⁴ This opposite effect on intestinal inflammation evokes the question about the underlying mechanism(s). We found that PTPN22 expression is reduced in patients with both UC and CD, with a dominant downregulation in CD68⁺ mononuclear cells. However, only a slight difference in PTPN22 expression in T or B cells was observed,²⁷ indicating an important role of PTPN22 in the myeloid cell compartment for maintaining intestinal homeostasis (Fig. 2).

PTPN22 in Experimental Colitis

Although several association studies revealed a relation between PTPN22 and IBD susceptibility,^{4,29} only a few studies addressed its functional role during intestinal inflammation. One study showed that in a T-cell mediated colitis model, where naive T cells were transferred into immune-deficient mice, naive PTPN22-deficient T cells aggravated intestinal inflammation and tissue damage.²³ Interestingly, the transfer of PTPN22-deficient regulatory T cells—but not the transfer of wild-type regulatory T cells—rescued this phenotype.²³ This effect is attributed to increased suppressive competence of PTPN22-deficient regulatory T cells.²³ A recent study further revealed that PTPN22-deficient naive T cells proliferate faster, acquire full effector functions, and loose self-tolerance in immune deficient hosts.³⁰ This might

explain how PTPN22-deficient T cells are able to mediate increased inflammation when injected into lymphopenic mice.

The important protective role of PTPN22 during intestinal inflammation has further been confirmed in 2 independent studies using a dextran-sodium sulfate (DSS)-induced model of acute colitis. In both studies, loss of PTPN22 resulted in increased reaction to DSS with aggravated weight loss and enhanced colitis severity.^{18,28} Enhanced colitis susceptibility has been attributed to altered TLR4 signaling and type I interferon production in one study,¹⁸ or altered macrophage polarization in the other report,²⁸ although the cell-type specific contribution to intestinal inflammation has not been addressed directly. We further observed that PTPN22 deficiency results in reduced myelin peroxidase activation and decreased granulocyte infiltration into the inflamed colon during DSS colitis (Spalinger MR, et al, unpublished data, 2014), suggesting a role for PTPN22 in mediating granulocyte function/recruitment.

As PTPN22 expression is reduced mainly in myeloid cells within the intestine of patients with IBD,²⁷ transfer of PTPN22-deficient T cells, or colitis induction in a setting where PTPN22 is missing in all cells, might however be an incomplete approach to address PTPN22 function during intestinal inflammation. Additional *in vivo* studies including PTPN22 deficiency specifically in nonlymphoid immune cells would greatly improve our general understanding on the role of PTPN22 in IBD.

In summary, patients with IBD feature have decreased levels of PTPN22 in myeloid cells in the intestine. Several studies demonstrated, that loss of PTPN22 in myeloid cells changes the response toward proinflammatory cytokines and bacterial products. Hence, reduction of PTPN22, as observed in patients with IBD importantly affects the cytokine balance within the inflamed intestine, ultimately promoting disease persistence and progression (Fig. 3).

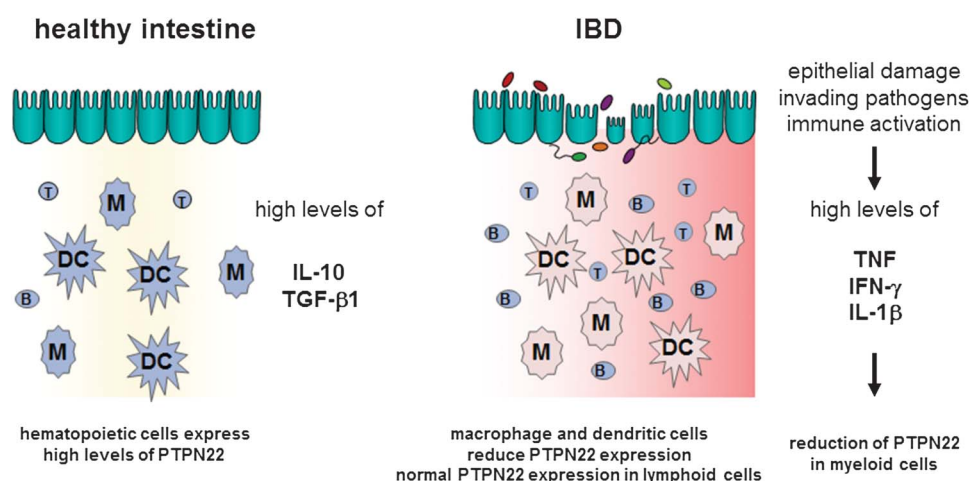


FIGURE 2. Reduced PTPN22 expression in patients with IBD. In the healthy intestine, high levels of IL-10 and TGF-β result in high expression of PTPN22 in all immune cells. On epithelial damage, the proinflammatory cytokine milieu results in reduced PTPN22 expression specifically in cells of the myeloid lineage, whereas lymphoid cells still express high amounts of PTPN22. B, B cell; DC, dendritic cell; IFN, interferon; M/MF, macrophage; M1, inflammatory macrophage; T, T cell.

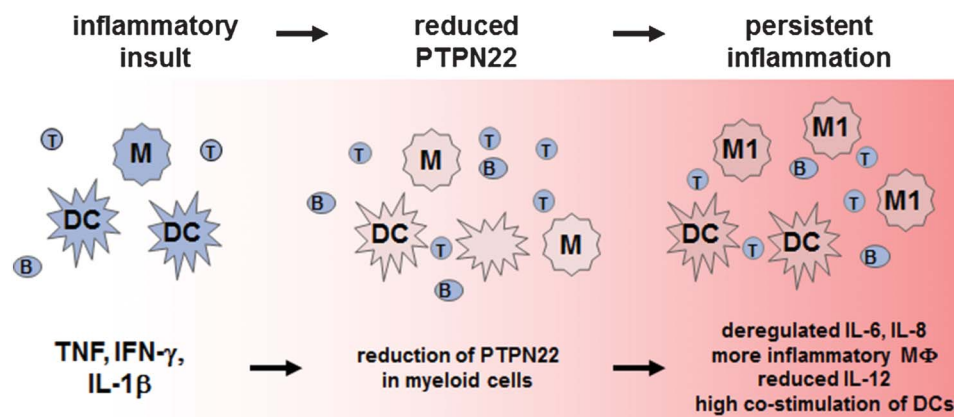


FIGURE 3. Proposed mechanism, how PTPN22 is involved in IBD pathogenesis. Inflammatory insults result in high levels of TNF, IFN- γ and IL-1 β . TNF and IL-1 β reduce PTPN22 expression in myeloid immune cells. On loss of PTPN22, monocytes/macrophages secrete increased levels of IL-6 and IL-8 and differentiate preferentially into proinflammatory M1 macrophages. The increased expression of costimulatory molecules on DC results in an enhanced activation of the adaptive immune system. B, B cell; DC, dendritic cell; IFN, interferon; M/MF, macrophage; M1, inflammatory macrophage; T, T cell.

Protein Tyrosine Phosphatase Non-receptor Type 2

The protein tyrosine phosphatase non-receptor type 2 (PTPN2) gene encodes the tyrosine phosphatase PTPN2, also known as T-cell protein tyrosine phosphatase. PTPN2 was named due to being originally cloned from a T-cell cDNA library. However, it is ubiquitously expressed.^{31,32} In humans there are 2 functional variants of the PTPN2 enzyme that occur through alternative PTPN2 splicing. The larger 48 kD form is restricted to the endoplasmic reticulum by a hydrophobic C-terminus that masks a nuclear localization sequence. The more enzymatically active 45 kD variant lacks the hydrophobic C-terminus and can therefore transit to the nucleus through the nuclear localization sequence and is thus regarded as the mobile form of PTPN2.^{33,34} Indeed, in response to an appropriate stimulus the 45 kD form can also exit the nucleus and dephosphorylate target substrates in the cytoplasm and at the plasma membrane.³⁵ PTPN2 is capable of dephosphorylating many protein tyrosine kinases such as the insulin receptor, epidermal growth factor receptor (EGFR), Src family kinases as well as several janus kinase (JAK) and STAT family members.^{35–41} The PTPN2 gene has emerged as a site of distinct clinical significance due to the association of a number of SNPs in the PTPN2 locus 18p11 with chronic inflammatory conditions such as CD, UC, T1D, and celiac disease.^{42–44} The rs2542151 SNP has been the most widely identified PTPN2 SNP associated with IBD. It was first reported in the defining Wellcome Trust Case Control Consortium article published in 2007 demonstrating a link with CD.⁴⁵ Follow-up studies confirmed this association and also identified links between the rs2542151 SNP with CD and UC.^{43,46–50} Additional SNPs in the PTPN2 gene locus have also been associated with IBD and disease outcomes. This includes the rs7234029 SNP that has a potential association with a stricturing disease phenotype in CD subjects and may be linked to early onset CD and UC.⁵¹ The rs1893217 SNP was originally

associated with T1D; however, it has emerged as a candidate SNP in both adult and early-onset pediatric CD and UC, although the effect of this SNP on the PTPN2 gene and function remains to be determined.^{42,43,52} Nevertheless, the first study points toward the presence of a loss-of-function PTPN2 protein in variant carrying cells.⁵³

PTPN2 and Barrier Function Regulation

With respect to PTPN2 expression in IBD, colonic PTPN2 mRNA and protein expression is increased in active CD with expression being most prominent in the epithelium.^{54,55} These findings were supported by another study that also showed increased PTPN2 protein expression in intestinal tissues (colon and ileum) from patients with active CD.⁵⁶ Consistent with this, we have shown that in intestinal epithelial cell (IEC) lines, the IBD-associated inflammatory cytokines, IFN- γ , and tumor necrosis factor (TNF) are capable of increasing expression of PTPN2.^{54,55} This suggests that these inflammatory cytokines induce expression of a negative regulator of their own signaling in an apparent negative feedback loop. Although expression of PTPN2 is increased in CD, the impact of noncoding SNPs seems to manifest in a loss of enzymatic activity or efficacy, although the full impact of individual SNPs on PTPN2 expression and function has yet to be fully determined. A loss of PTPN2 expression has been shown to have dramatic consequences for IECs and their ability to form an effective barrier. siRNA knockdown of PTPN2 resulted in accentuated barrier defects induced by IFN- γ treatment of IEC.⁵⁴ This included a greater decrease in transepithelial electrical resistance in response to IFN- γ coupled with a pronounced increase in expression of the cation-selective pore-forming molecule, claudin-2.⁵⁴ Claudin-2 expression is elevated in colonic tissues in patients with IBD, especially UC, and functionally, this could contribute to symptoms of disease by permitting increased paracellular passage of sodium ions into the intestinal lumen

thus tangibly contribute to intestinal fluid loss associated with IBD.^{57–59} In addition to a further decrease in transepithelial electrical resistance, PTPN2-deficient cells also displayed increased macromolecule permeability following IFN- γ treatment as determined by increased apical–basolateral passage of FITC-dextran across polarized IEC monolayers.⁵⁴ Due to the width of the pore size generated by claudin-2 being insufficient to permit passage of FITC-dextran (10 kD), this strongly suggests that additional mechanisms capable of modifying tight junction components responsible for regulation of macromolecule permeability are recruited by IFN- γ in cells lacking PTPN2.⁶⁰ Conclusive evidence of this has not yet been determined as PTPN2 knockdown that did not cause further decreases in expression of the tight junction proteins occludin or zonula occludens-1 by IFN- γ , whereas a possible influence of PTPN2 on relocalization of tight junction proteins has not been investigated.⁵⁴ These data suggest that PTPN2 plays an important role in protecting intestinal epithelial barrier function. A protective role for PTPN2 in intestinal barrier function has also been indicated in vivo. Ptpn2 knockout mice exhibit a pronounced phenotype at 3 to 5 weeks of age that is characterized by systemic inflammation, hematopoietic defects, increased levels of proinflammatory cytokines, splenomegaly, and diarrhea. The severity and kinetics of the inflammation in mice are strain dependent with an accelerated morbidity for mice on a Balb/c background compared with mice on a C57BL/6 background.^{61,62} Bone marrow chimeric studies indicated that the inflammation and mortality in mice on the Balb/c background were governed by loss of Ptpn2 in the nonhematopoietic compartment.⁶³ Studies with heterozygous Ptpn2-deficient mice showed that these mice have no overt inflammatory phenotype and exhibit normal survival rates. However, they did exhibit increased sensitivity to DSS-induced colitis suggesting that Ptpn2/PTPN2 deficiency increases the susceptibility of the intestine to agents that disrupt the epithelial barrier.⁶⁴

One additional area of epithelial function that can contribute to the broader concept of “barrier function” is appropriate regulation of electrolyte transport. This is essential for absorption and secretion of electrolytes and fluids as well as being responsible for the absorption of nutrients, maintenance of luminal pH, and preserving the sterility of intestinal crypts.⁶⁵ Epithelial electrolyte transport is suppressed in IBD, and this is believed to contribute to overall fluid loss through decreased absorptive capacity, as well as creating an environment conducive to increased bacterial interactions with the intestinal epithelium.^{66–68} PTPN2 has been shown to play a role in regulating fluid secretion. Specifically, PTPN2 knockdown in T₈₄ colonic epithelial cells accentuated epidermal growth factor inhibition of Ca²⁺-stimulated chloride secretion thus promoting EGFR suppression of electrolyte secretion.⁶⁹ Thus, it is possible that PTPN2 mutations resulting in a loss of enzymatic activity could encourage elevated or prolonged EGFR phosphorylation and exacerbate overall dysregulation of intestinal fluid homeostasis.

As discussed later in this article, loss of PTPN2 expression also has consequences for other aspects of innate and adaptive

immunity including autophagy and T-cell function.^{53,70} Given that PTPN2 represents a point of convergence for multiple aspects of intestinal homeostasis, it may therefore play a key role in multiple IBD-associated physiological events and thus, like other IBD candidate genes, make a greater cumulative contribution to IBD pathogenesis than suggested by the genetic prevalence of PTPN2 SNPs in sampled populations. Therefore, clinically relevant loss-of-function mutations in the PTPN2 gene may contribute, at least in part, to the development of IBD and other chronic inflammatory intestinal diseases through a compromised epithelial barrier. Indeed, given that increased intestinal permeability is a feature of IBD, T1D, and celiac disease, this may be one avenue to explore commonality in the etiology of these conditions arising from PTPN2 mutations (Fig. 4).

PTPN2 and Innate Immune System

Studies with Ptpn2^{−/−} mice confirmed that PTPN2 is a key negative regulator of cytokine signaling. These animals develop progressive systemic inflammatory disease as demonstrated by chronic myocarditis, gastritis, nephritis, and sialadenitis as well as elevated serum levels of IFN- γ , IL-12, TNF, and nitric oxide. These cytokines seem to be mostly produced by mononuclear cells. Interestingly, PTPN2-deficient mice also present increased sensitivity to the bacterial wall component, lipopolysaccharide, in vivo and in vitro cultured macrophages from Ptpn2^{−/−} mice are hypersensitive to lipopolysaccharide.⁶³

Recent data have demonstrated that PTPN2 regulates IFN- γ -induced signaling and effects in cell models of chronic intestinal inflammation. Treatment of human IEC with IFN- γ increases PTPN2 mRNA and protein levels, elevates enzymatic PTPN2 activity, and causes cytoplasmic accumulation of PTPN2. Similar as in fibroblasts, these effects are mediated through the cellular energy sensor, adenosine monophosphate-activated protein

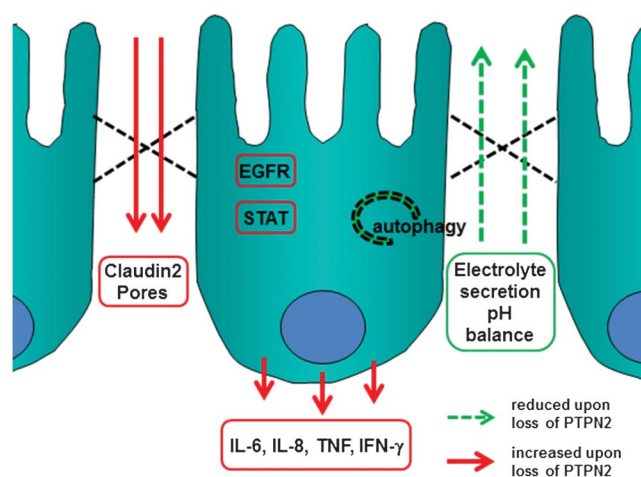


FIGURE 4. PTPN2 functions in IEC. Loss of PTPN2 or presence of PTPN2 variants results in reduced barrier function, and suppression of electrolyte secretion resulting in a disturbed liquid/electrolyte balance. Furthermore, PTPN2 deficiency results in enhanced autophagy and increased secretion of proinflammatory cytokines.

kinase. Knockdown studies using PTPN2-specific siRNA constructs revealed that dephosphorylation of STAT1+3 is dependent on PTPN2 in these cells.⁵⁴

In PTPN2-deficient human THP-1 monocytic cells IFN γ induced activity of the MAPK-subunit p38. Additionally, secretion of monocyte chemoattractant protein 1 (MCP-1) and IL-6 were enhanced.⁷¹ PTPN2 also regulates signaling responses to the bacterial wall component MDP in human monocytes finally resulting in enhanced secretion of IFN- γ but reduced secretion of IL-8 and TNF from these cells. This might be due to the fact that dysfunction of PTPN2 in human monocytes causes enhanced MAPK signaling in response to MDP. Of note, PTPN2 dysfunction also resulted in enhanced cleavage of caspase-1 and increased IL-1 β secretion, indicative of increased inflammasome activation, in response to MDP.⁵³ Further studies demonstrated that TNF induces PTPN2 protein and mRNA levels in human IEC through a nuclear factor κ B-dependent mechanism. PTPN2 in turn regulates TNF-induced ERK- and p38-MAPK activity as well as IL-6 and IL-8 secretion.⁵⁵ A further study also demonstrated that PTPN2 controls TNF-induced IL-6 secretion in mouse embryonic fibroblasts.³⁹ These findings strongly suggest that PTPN2 is crucial for maintaining intestinal homeostasis and controlling cytokine secretion from IECs, fibroblasts, and mononuclear cells.

PTPN2 Regulates Autophagosome Formation

Autophagy is a fundamental process for bulk degradation of cytoplasmic compartments, damaged organelles and/or misfolded proteins. In autophagosomes, these structures are sequestered into double-membrane-enclosed vesicles and delivered to lysosomes for final degradation.^{72–74} Autophagy is activated by stress conditions, such as starvation or hypoxia, and dysfunction of autophagy has been implicated in numerous pathologies, such as cancer or neurodegeneration.⁷⁵ Autophagy is also critically involved in host defense against intracellular pathogens, such as *Listeria monocytogenes* or *Salmonella typhimurium*.^{76–78} This observation further supports the hypothesis that abnormal immune responses to luminal bacteria or bacterial antigens play an essential role for the manifestation of CD. Previous data have well demonstrated that presence of genetic variations within autophagy genes results in a defective bacterial handling, prolonged intracellular survival of pathogenic bacteria, and an uncontrolled inflammatory situation.

Recent data demonstrated that PTPN2 not only regulates the cytokine-induced activation and expression of autophagy-related molecules but is also involved in the regulation of autophagosome formation in IEC.⁷⁹ siRNA-induced knockout of PTPN2 in IEC inhibits the expression of several autophagy-associated molecules, including beclin-1, ATG5, ATG7, ATG12, ATG16L1, and IRGM in response to IFN- γ and TNF cotreatment. Of note, reduced protein levels of all of these autophagy markers have also been observed in intestinal tissue samples derived from patients with active CD when compared with tissue samples from non-IBD controls.⁷⁹

On a functional level, loss of PTPN2 in human IEC completely abrogated the increase in LC3B-II protein levels and the formation of LC3B-positive (LC3B⁺) subcellular structures in the cells, indicating reduced autophagosome formation, in response to TNF and IFN- γ cotreatment. Furthermore, PTPN2-deficient cells feature only a small number of LC3B⁺ vesicles overall and TNF and IFN- γ cotreatment caused the formation of fewer, but larger LC3B⁺ vacuoles that were localized close to cell borders.⁷⁹ The appearance of such abnormal large autophagic vacuoles has been regarded as a marker of an ineffective formation of dysfunctional autophagosomes due to a defective autophagy process in these cells.⁸⁰ Similar likely dysfunctional autophagosomes were observed in PTPN2-deficient MDP-treated human monocytes.⁵³ Interestingly, the effects of PTPN2 on autophagosome formation seemed to be mediated by controlling the phosphorylation status of the EGFR and subsequently of phosphatidylinositol 3-kinase (PI3K), Akt, and molecular target of rapamycin activity.⁷⁹

Using primary colonic lamina propria fibroblasts (CLPF) isolated from patients with CD featuring either the PTPN2-WT or the CD-associated PTPN2 variant, rs2542151, it was demonstrated that presence of the disease-associated PTPN2 variant exerts similar effects than siRNA-induced loss of PTPN2 expression.⁷⁹ In particular, CLPFs featuring the CD-associated PTPN2 variant revealed reduced basal levels of PTPN2 protein when compared with PTPN2-WT fibroblasts and the increase in PTPN2 protein in response to TNF and IFN- γ treatment was absent in the variant cells. As in IEC, PTPN2 dysfunction also prevented the cytokine-induced rise in the expression of autophagy markers, such as IRGM and also resulted in diminished formation of autophagosomes in PTPN2-variant carrying CLPF.⁷⁹

Impaired autophagy has been described to result in defective handling of invading bacteria and a defective handling of luminal and/or invading bacteria might critically contribute to the onset of IBD. Therefore, the question arises whether PTPN2 dysfunction might result in ineffective clearance of invading bacteria due to the altered autophagosome formation. Interestingly, using GFP-labeled *L. monocytogenes*, it has been demonstrated that defective PTPN2 function results in enhanced levels of intracellular *L. monocytogenes* and reduced numbers of autophagosome as well as of LC3B-II protein levels in IEC. These findings were fully confirmed using CLPF from patients being either PTPN2 wild type or variant.⁷⁹ Presence of the CD-associated PTPN2 variant in CLPF caused similar effects with respect to autophagosome formation and the number of invading bacteria as PTPN2 deficiency in IEC. In PTPN2-variant CLPF, LC3B-staining was clearly less detectable while a strong GFP⁺ staining, suggestive for a large number of intracellular *L. monocytogenes*, was observed.⁷⁹ These data demonstrate that dysfunction of PTPN2 results in impaired autophagosome formation and defective handling of invading bacteria and suggest how presence of the CD-associated PTPN2 variant within intestinal cells could critically contribute to the onset of IBD by causing a defective function of the innate immune system.

PTPN2 and Adaptive Immune System

PTPN2 had initially been termed as T-cell protein tyrosine phosphatase, which indicates that it was first detected and characterized in the T-cell subset of the adaptive immune system.^{81,82} Subsequently, a number of functions for PTPN2 in the adaptive immune system have been described in the following years. T cells express PTPN2 at especially high levels indicating a specific role in this cell type.⁸³ But also, B-cell development is crucially regulated by PTPN2: The knockdown of PTPN2 leads, besides other phenotypic changes in the respective animals, to an early bone marrow B-cell deficiency, which is caused by a block of the transition from pre-B cells to immature B cells.⁸³ This is associated with the secretion of high amounts of IFN- γ , which in turn is followed by phosphorylation of STAT molecules in the pre-B-cell compartment.⁸⁴ Normally, an important function of PTPN2 is to dephosphorylate STAT1 leading to its inactivation.⁴⁰ On ligand binding, cytokine-receptor-associated kinases phosphorylate STAT molecules, which subsequently dimerize and translocate to the nucleus, where they act as transcription factors. PTPN2 counteracts the activity of receptor-associated kinases by dephosphorylation of STAT molecules, ultimately repressing cytokine signaling.⁴¹

Similar to the innate immune system, PTPN2 has an anti-inflammatory role in the adaptive immune system, both in the T- and B-cell compartment. Not surprisingly, mice homozygous deficient of PTPN2 suffer from uncontrolled inflammation and die 3 to 5 weeks after birth.⁶¹ They show severe alterations in organs of the adaptive immune system including splenomegaly and lymphadenopathy including impaired T- and B-cell functions.⁶¹ These data indicate an important role of PTPN2 in the adaptive immune system and in the maturation of function of B and T cells and subsequently in autoimmunity are supported by data from human autoimmune diseases. In GWAS in patient cohorts with different autoimmune diseases, SNPs of PTPN2 have been linked with the development of the disease such as T1D and CD.⁷⁰

Besides B- and T-cell maturation as well as STAT1 dephosphorylation, this may be caused by the important role of PTPN2 that has as a key negative regulator of TCR signaling.⁷⁰ PTPN2 dephosphorylates and inactivates Src family kinases to regulate T-cell responses.⁷⁰ By interfering with TCR signaling, PTPN2 attenuates T-cell activation and proliferation and limits antigen-induced responses. Controlled TCR activation and signaling is important for the priming of cytotoxic CD8⁺ T cells to pathogens and pathologic antigens on one hand, as well as mediating peripheral tolerance to self-antigens or commensal microbes in the gut on the other. PTPN2-deficient antigen stimulated CD8⁺ T cells are no longer self-tolerant and are able to destroy cells such as pancreatic β cells in an autoreactive manner finally resulting in the development of diabetes even in the absence of CD4⁺ T-helper cells.⁸⁵ Thus, PTPN2 variants can redirect a normally tolerogenic CD8⁺ T-cell response into an autoreactive and destructive response.

In addition, a deficiency of PTPN2 was reported to enhance naive T-cell responses to low-affinity ligands.⁷⁰ This may partially be associated with the fact that STAT3 and STAT5 are further substrates for dephosphorylation by PTPN2 (Fig. 5). Wiede et al⁷⁰ reported that in the periphery PTPN2 deficiency resulted in a memory phenotype of CD4⁺ T cells. In their mouse model where PTPN2 lacks specifically in the T-cell compartment, the number of T cells with an effector/memory phenotype increased progressively from 4 to 12 weeks of age, which was paralleled by a decrease in naive T-cell numbers. This may lead to a selection of high-affinity, potentially self-reactive T cells, which would represent another pathway of autoimmune disease induction by a lack of PTPN2 function. In contrast, a lack of PTPN2 function does not seem to influence the number of regulatory T cells and does not interfere with regulatory T-cell function.

The increase in secretion of proinflammatory cytokines found in other cell types certainly can also be found in T cells. Misbalanced JAK and STAT signaling may play an important role

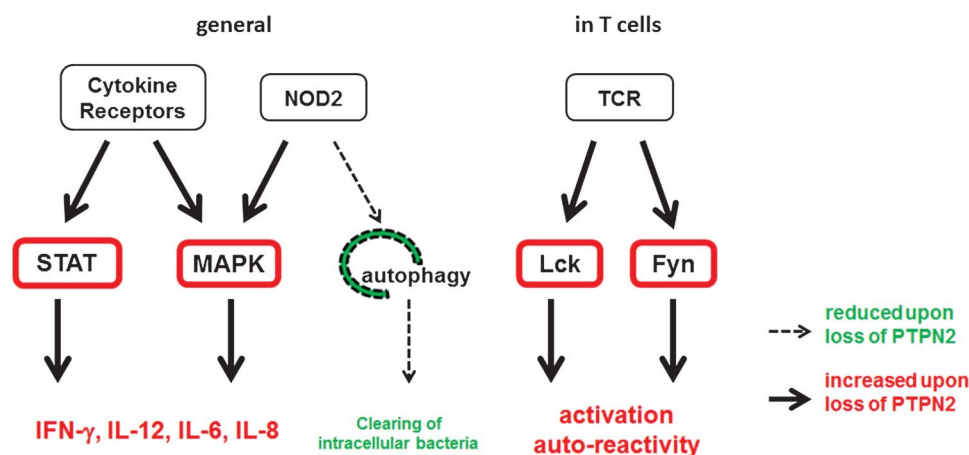


FIGURE 5. Signaling pathways regulated by PTPN2. PTPN2 dephosphorylates STAT1, STAT3, and STAT5 to reduce cytokine signaling. In addition, loss of PTPN2 results in enhanced MAPK activation and decreased autophagy induction. In T cells, PTPN2 counteracts TCR-associated kinases and thereby controls the reaction toward antigens maintaining tolerance toward self-antigens. Lck, lymphocyte tyrosine kinase; NOD2, nucleotide oligomerization domain containing protein 2.

here. An upregulation of IFN- γ secretion, IL-12 secretion, and other cytokines may play an important role for T-cell activation.⁸³ Additionally, it has been demonstrated that PTPN2 is elevated in naive T cells that leave the thymus. PTPN2-deficient CD8⁺ T cells undergo rapid lymphopenia-induced proliferation after transfer into lymphopenic hosts acquiring characteristics of antigen-experienced effector T cells. This increase in lymphopenia-induced proliferation is associated with elevated TCR-dependent, but not IL-7-dependent responses. This finally results in aberrant TCR function and the development of autoimmunity⁸⁶ (Fig. 6).

Despite the important role of PTPN2 and the finding of relevant variants in autoimmune diseases such as CD and UC, a detailed analysis of modifications of T-cell functions by those variants or a complete lack of PTPN2 has not been performed yet. Animal models may not be optimally suited as there are complex interactions between the immune cells and also between adaptive and innate immune mechanisms.

Role for Other PTPNs in Intestinal Inflammation

In addition to PTPN2 and PTPN22, some other PTPNs have also been associated with intestinal inflammation. In particular, PTPN1 (PTP1B), PTPN6 (SHP-1), and PTPN11

(SHP-2) have already been demonstrated to play a role for the development of intestinal inflammation.

PTP1B is a key molecule in modulating low-degree inflammatory conditions such as diabetes. A recent study demonstrated that PTP1B deficiency ameliorates DSS-induced murine experimental colitis through expanding CD11b⁺Gr-1⁺ myeloid-derived suppressor cells. PTP1B null mice demonstrate greater resistance to DSS-induced colitis, as reflected by slower weight loss, greater survival rates, and decreased macrophage infiltration into the colon. This resistance of PTP1B-deficient mice to DSS-induced colitis is based on the expansion of myeloid-derived suppressor cells regulating cytokine secretion and activation of signaling molecules.⁸⁷

A further study demonstrated a significant association between PTPN6 and UC in a collective of Tunisian patients with IBD.⁸⁸ On a functional level, PTPN6 has been identified as a critical regulator of Th17 development. Genetically or pharmacologically caused dysfunction of PTPN6 activity in T cells makes these cells hyperresponsive to stimulation through IL-6 and IL-21. Additionally, PTPN6 controls cytokine-induced phosphorylation of STAT3 in primary CD4⁺ T-helper cells. These data suggest a role for PTPN6 in the pathogenesis of IBD by modulating Th17 cell development.⁸⁹

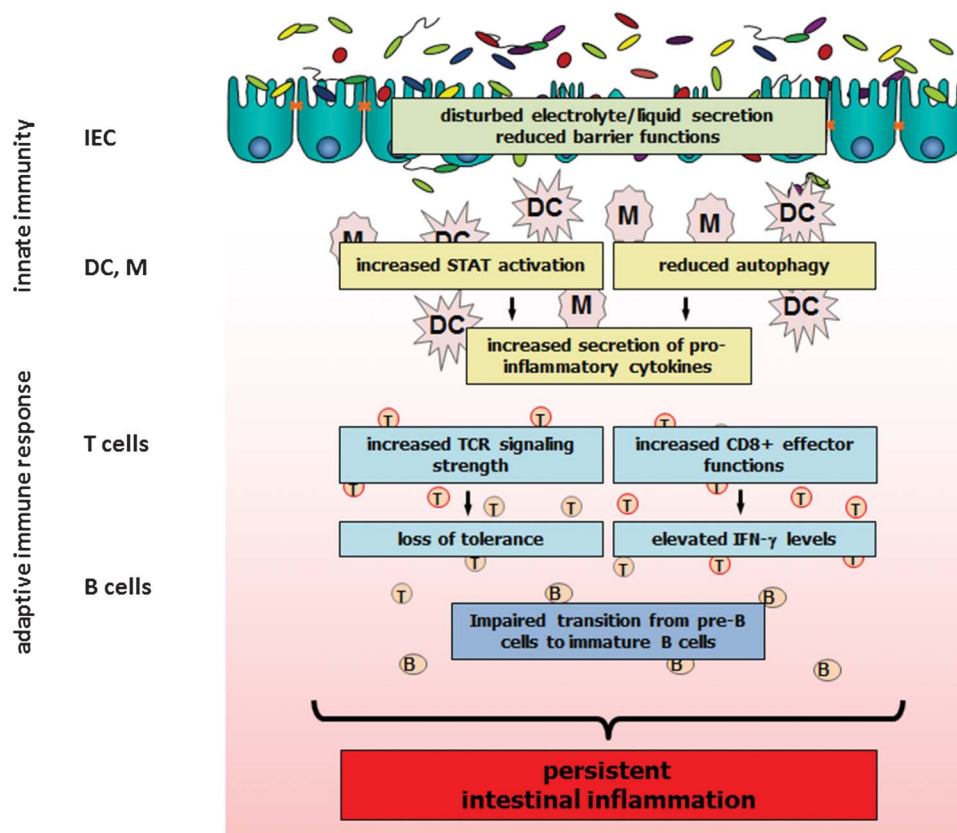


FIGURE 6. The effect of loss of (functional) PTPN2 during intestinal inflammation. Loss of PTPN2 in experimental models or the presence of PTPN2 variants in humans results in several disease driving mechanisms ultimately maintaining intestinal disease. B, B cell; DC, dendritic cell; M, macrophage; T, T cell.

PTPN11 is a ubiquitously expressed cytoplasmic protein tyrosine phosphatase. Genetic associations exist between UC, but not CD, and PTPN11. The Hap 1 haplotype and its homozygous Hap 1/Hap 1 diplotype of PTPN11 is significantly increased in patients with UC compared with control subjects.⁹⁰ The expression of PTPN11 protein in the intestinal tissue of patients with UC is reduced.⁹¹ On a functional level, it has been demonstrated that pharmacologically induced interaction between PTPN11 and cytosolic STAT1, what finally prevents the recruitment of STAT1 to the IFN- γ receptor and inhibits STAT1 signaling, results in reduced Th1 cytokine production and an improvement in 2,4,6-trinitrobenzene sulfonic acid-induced colitis in mice. Inhibition of PTPN11, vice versa, worsens colitis symptoms in vivo.⁹²

Mice featuring an IEC-specific PTPN11 deletion feature growth retardation and rapidly develop clinical and histological signs of severe colitis. These animals also feature decreased expression of claudins accompanied by enhanced intestinal permeability and an upregulation of STAT3 and nuclear factor κ B activity as well as an induction of several epithelial chemokines and cytokines. Interestingly, the development of colitis in PTPN11 IEC specific knockout mice was remarkably impaired by antibiotic treatment. A further study demonstrated that the number of goblet cells in both the small intestine and colon of these mice is clearly reduced compared with control mice. Furthermore, the PTPN11-deficient mice showed marked impairment of both, IEC migration along the crypt-villus axis in the small intestine and development of intestinal organoids from isolated crypts.^{91,93} These findings suggest that intestinal epithelial PTPN11 might play a critical role for protecting the intestinal epithelium and thereby for the prevention of gut inflammation.

SUMMARY

PTPs play a crucial role for regulating intracellular signaling events. Recent GWAS have associated a number of PTPs with the onset of IBD. Evolving evidence emerges that expression levels of PTPs, mainly PTPN2, PTPN22, and PTPN11 are altered in actively inflamed intestinal tissue. Furthermore, the PTPs seem to be critical for protecting intestinal epithelial barrier function, regulating innate and adaptive immune responses, and finally for maintaining intestinal homeostasis. Dysfunction of those PTPs results in aberrant and uncontrolled immune responses that result in chronic inflammatory conditions. This way, it becomes more and more evident that dysfunction of PTPs displays an important factor in the pathogenesis of chronic intestinal inflammation, in particular IBD.

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